

Review Article

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## Review on Exogenous Hormone Administration in Aquaculture

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### ABSTRACT

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Aquaculture is the fastest expanding sector of animal food production and has a lot of potential as a long term solution for global food security. The ability to controlling growth, reproduction and sex is crucial aspects of aquaculture for increasing aquaculture production. Many natural and synthetic hormones are used in aquaculture. This present review focused on the use of various hormones in fish breeding, sex reversal, growth promotion and immune boosting in aquaculture. In fish breeding, pituitary gland extract and gonadotropic releasing hormone analogues are commonly used to induce spawning in captive condition and produce good quantity and quality seed throughout the year. Estrogen and androgen are the mostly used in sex reversal of fish when one gender of species has the potential to grow faster and bigger than other gender. Administration of thyroid hormone and tryptophan helps fish grow bigger and less stressed. In order to increase food safety and limit environmental impact, these hormones should be used with caution in aquaculture. In order to increase food safety and limit environmental impact, these hormones should be used with caution in aquaculture. In order to increase food safety and limit environmental impact, these hormones should be used with caution in aquaculture.

### Introduction

Hormones are chemical messengers responsible for the communication between different types of cells that recognize their identity and function through receptors, which are protein structures specialized in molecular recognition (Hafez *et al.*, 2000). Several biochemical processes that result in specific biological responses happen after the proximity and the hormone-receptor contact (Reis-Filho *et al.*, 2006). Hormones in aquaculture are used for artificial reproduction and sex reversal (Hoga *et al.*,

2018). The first one maintains the production cycle by continuously producing seeds. The second is used when the growth rate or weight gain differs between males and females. This difference between genders is common in teleost fish and usually occurs during puberty (Taranger *et al.*, 2010). Additionally, some hormones in aquaculture are used to develop growth (Cristea *et al.*, 2012), increase immunity and reduce stress (Campbell *et al.*, 2021). Nevertheless, using hormonal products in fish farming can have harmful consequences, such as potential risks to human and environmental health related to

hormone-dependent parameters. Moreover, its use outside of good animal husbandry practices may affect not only the fish farming production system but also the commercialization of this food commodity. The most important sex hormones used in fish farming are estrogens and androgens, which can be of natural origin or synthesized or produced in the laboratory (Pandian *et al.*, 2003). The present article will emphasise the hormones used to induce breeding, and sex reversal to reduce stress, and to increase immunity and growth.

### **Hormones to Induce Spawning in Fish**

The sustainability of commercial aquaculture output depends on the control of reproductive function in captivity. For many fish, this may be done by adjusting photoperiod, water temperature, or spawning substrate (Maylonas *et al.*, 2010). Reproduction in fish is controlled by the hormones released by the brain, pituitary and gonads. Hence, hormonal manipulation to induce spawning is a technique in aquaculture (Mousavi *et al.*, 2012). The hypothalamus-pituitary-gonadal axis is the pathway for gamete formation, final oocyte maturation, and ovulation in fish (Mylonas *et al.*, 2010). Several hormones are used in spawning includes Carp pituitary extract (CPE) (Wang *et al.*, 2009), Channel cat fish pituitary (CCP) (Dunham and Masser, 2012), Growth hormone (GH), Human chorionic Gonadotropin (HCG) (Levavi-Sivan *et al.*, 2004), Ovaprim (Targońska *et al.*, 2010), Ovatile (Sahoo *et al.*, 2005), Ovapel (Surnar *et al.*, 2015), Salmon gonadotropin releasing hormone analog (sGnRH $\alpha$ ) (Heyrati *et al.*, 2007) and Mammalian luteinizing hormone releasing hormone analog (mLHRH) (Berlinsky *et al.*, 2005). Hormones used in spawning can be used alone and in combination. The induced breeding approach is used to reduce reproductive failure in fish (Mosha, 2018) and to provide fish seed throughout the year (Ochokwu *et al.*, 2015).

The first step for hormone-induced spawning is determining the type of hormone suitable for the fish species of interest. The efficacy of hormones is often influenced by species, physiological status, and

hormone dose under consideration. A critical step in the hormone-induction technique used to spawn food fish is measuring exact hormone dosages depending on the prescribed amount. In addition, it is desirable to know the number of fish, the weight of the fish, the volume of hormone solution to inject, the number of injections, and the injection schedule (Rottmann *et al.*, 2001). It is challenging to choose suitable hormone to induce spawning of fish. For instance, there may be variations in the species response, differences attributed to the stage of maturity, or even the time of hormone injection. It is more crucial to adjust the dose than to worry about choosing the right hormone because a small increase in dosage may compensate for a lower activity for a particular species. Hence, cost-effectiveness is essential when choosing a hormone based on its effectiveness and potency.

### **Human Chorionic Gonadotropin (HCG)**

Human chorionic gonadotropin is discovered in the blood of pregnant women. It is the only US FDA-approved hormone for inducing spawning in fish and is marketed as Chorulon (Elakkanai *et al.*, 2015). However, it is ineffective in many fish species and is not commonly used. This hormone is given through intraperitoneal injection. HCG administration increases the concentration of gonadotropin in the blood to induce maturation and ovulation (Rottmann *et al.*, 2001). Generally, HCG is administered in two doses.

First dosage is a primary dose (20 per cent), and second dosage called resolving dose (80 per cent), which is administered after an optimal period, depending upon fish species, temperature, and condition of the fish. HCG has been used to induce spawning in many fish species, including magur, singhi and african catfish (Kahkesh *et al.*, 2010; Haniffa and Sridhar, 2002; Sahoo *et al.*, 2007).

### **Carp Pituitary Extract (CPE)**

In the past, the most widely utilised spawning hormone in aquaculture was carp pituitary extract

(CPE). Carp pituitary hormone can acquire as powder and whole gland. Carp pituitary extract increases the gonadotropin level in the blood plasma of a fish to stimulate final maturation leading to ovulation under captivity (Zamri *et al.*, 2022). The maximum suggested dosage is 4.5 milligrams per pound (10 mg/kg) body weight to induce spawn by intraperitoneal injections (Rottmann *et al.*, 2001).

CPE has been used to induce spawning in fish species, including common carp, channel catfish, rainbow trout, Indian major carp, and Chinese carp (Kahkesh *et al.*, 2010).

### **Channel Catfish Pituitary (CCP)**

Channel catfish pituitary is obtained from the pituitaries of mature channel catfish. The pituitaries are processed and made available for hormone-induced spawning, similar to CPE. This hormone facilitates final oocyte maturation and ovulation in gravid fish (Senthilkumaran and Kar, 2021).

Although catfish pituitaries are easily accessible for induced breeding, their present use in fish spawning in commercial hatcheries appears to be constrained by their cost and inconsistent quality. The maximum suggested dose is 4.5 mg/pound body weight (10 mg/kg) through multiple IP injections (Rottmann *et al.*, 2001).

### **Gonado Tropic Hormone (GTH)**

The analogues of sGnRHa (salmon gonadotropin-releasing hormone) and mLHRHa (mammalian luteinizing hormone-releasing hormone) act at a greater level in the HPG axis to increase the production of fish gonadotropin (Rottmann *et al.*, 2001). The hormone dose is drastically reduced compared to gonadotropic hormones, which are effective for induced spawning. GTH has been used to induce spawning in fish species, including Indian major carps, exotic carps, cod, loach, grouper and snapper (Garber *et al.*, 2009; Wang *et al.*, 2009; Kanemaru *et al.*, 2012).

### **Luteinizing Hormone Releasing Hormone Analog (LHRHa)**

Luteinizing hormone releasing hormone analog is a deca peptide having a 10-amino acid sequence Glu-His-Trp-Ser-Tyr-Gly-Ala-Leu-Arg-Pro-NH-CH<sub>2</sub>-CH<sub>3</sub> used to stimulate pituitary to release gonadotropins (Roy, 2016). Synthetic GnRHs /LHRHs are more potential than natural form. Additionally, LHRHa hormone induces spawning in a variety of fish species across the world due to lack of species specificity. The maximum hormone dose of LHRHa is 100 µg/ kg body weight. LHRHa is effective in inducing spawning in silver carp, common carp, mud carp, black carp, grass carp, milkfish, sea bass, cod and loach (Lee *et al.*, 1986; Peter *et al.*, 1988; Berlinsky *et al.*, 2005; Garber *et al.*, 2009).

### **Salmon Gonadotropin-Releasing Hormone Analog (sGnRHa)**

Marketed as OvaRH, sGnRHa can be used to induce food fish to spawn in hatcheries hormonally. This GnRH resembles mLHRHa but appears to have a better binding to pituitary receptors and an increased release of gonadotropin to induce maturation and ovulation in fish (Marino *et al.*, 2003). Generally, sGnRHa is more expensive than mLHRHa. However, sGnRHa is more potent, and the dose is often 6 to 7 times lower than mLHRHa. It is advisable to use sGnRHa instead of mLHRHa to induce the spawning of food fish (Rottmann *et al.*, 2001).

### **Ovaprim**

Syndel Laboratories, Canada, prepared the Ovaprim. Ovaprim contains 20µg of salmon GnRH and 10mg of domperidone (dopamine antagonist) per milliliter (Rottmann *et al.*, 2001). In India, trials with Ovaprim have given very encouraging results (Nandeeshha *et al.*, 1990, 1991). Ovaprim was used to successfully spawn mrigal (Kaula and Rishi, 1986). Nandeeshha *et al.*, (1990, 1991) reported satisfactory results in trials with Ovaprim. In a fish

seed hatchery, Khan *et al.*, (1992) reported that Ovaprim was used to successfully spawn Rohu and Mrigal. Additionally, ovaprim has been used in spawning of catfish, common carp, mrigal, sea bream and snaper (Watson *et al.*, 2009; Surnar *et al.*, 2015; Nuraini *et al.*, 2017; Mosha, 2018; Abbas *et al.*, 2019). However, a significant constraint in using Ovaprim is its high viscosity, which causes difficulty in injection. Its high cost is also a prohibitive factor.

### **Ovatide**

Another synthetic hormone, "Ovatide", has been manufactured by Mumbai-based Hemmo Pharma for the same purpose. The main ingredient of overtime is a synthetic peptide protein, which is analogous to naturally occurring gonadotropin-releasing hormone GnRH and dopamine antagonist. Ovatide composed of 20 µg of Salmon GnRH and 10 mg of domperidone per milliliter (Rottmann *et al.*, 2001). In contrast to Ovaprim, Ovatide is less expensive and viscous. Central Institute of Fisheries Education India conducted extensive field trials on induced fish breeding, including carp, using Ovatide in Madhya Pradesh, Andhra Pradesh, Haryana and Maharashtra (Khan *et al.*, 2006). Ovatide has been used to induce spawning in fishes includes grass carp, murrel (Marimuthu *et al.*, 2007), Indian major carps (Mishra *et al.*, 2001) and pabda (Dhawan and kaur *et al.*, 2004).

### **Hormones to Sex Reversal in Fish**

In aquaculture, sex reversal hormones are used to increase fish production when one sex of a species can grow faster and bigger than the other sex. Hormonal therapy during sex differentiation can result in sex reversal of fish. During the development of fish larvae, phenotype differentiation happens naturally, often earlier in females than males (Piferrer, 2001). Phenotype differentiation occurs naturally, generally earlier in females than in males, during the ontogeny of the fish larvae (Piferrer, 2001). This complex process can be manipulated using androgen and estrogen

hormones. Using hormones in fish farming for sex reversal aims to produce a mono-sex population to increase growth rate or weight gain. In order to get more uniform lots and prevent undesired breeding, it is preferable to rear the most profitable gender (Taranger *et al.*, 2010; Singh, 2013). Use of 17βestradiol, estradiolvalerate, 17α-methyl testosterone, or 17α-methyl dihydro testosterone (by immersion and diet technique) to produce an all-female population through direct and indirect feminization. On the other hand, androgen is used to masculinize females, producing no males (Piferrer, 2001). Females are produced due to the problem with early sexual maturity in male and the benefit of females growing more faster than males.

The synthetic androgen 17ot-methyltestosterone (MT), has been commonly used to transform genetic females into functional males in various species. More recently, mibolerone, 17ot-methyldihydrotestosterone (MDHT) (Piferrer *et al.*, 2001), trenbolone acetate (TEA), and other synthetic androgens have proven to be generally more effective than MT.

The administration of hormones for sex reversal treatment can be done using systemic (direct injection and Silastic implantation), immersion, or dietary supplementation (hormone incorporated in fish feed) (Pandian and Sheela, 1995).

Commercially, the most successful treatments use immersion and diet, as both methods reach many fish. In contrast, the systemic transfer method is expensive and requires technical ability to be applied to the fish. In the immersion technique, the dose administered affects the efficiency of hormonal treatments and other parameters, such as the type of hormone, water temperature and exposure time.

According to Pandian and Sheela (1995); Piferrer *et al.*, (2001), the administration of hormones through feed is more effective since it is simple to regulate and enables the use of optimum dose to induce sex reversal completely. The advantages of hormonal treatment are to ensure maximum growth, eliminate

early maturation in males and allow brood stock management. The drawback of sex reversal technique is existence of carcinogenic steroid residues that are harmful to the consumer health.

Hormonal induction of sex reversal can become stressful, resulting in low survival rates, delayed sexual maturity and reduced fish fertility. At the same time, high doses can lead to sterility, paradoxical sexual reversal and growth suppression. On a large scale, sexual reversal may become a polluting technique because more than 99% of hormones are metabolized and released within hours or days into the water.

### **Hormones to Reduce Stress in Fish**

Cortisol and ACTH (Adrenocorticotropic hormone) levels are affected by tryptophan. The most common stress marker in fish is circulating cortisol, which has been used to assess the mitigating effects of tryptophan in fish (Lepage *et al.*, 2002; Hoseini *et al.*, 2012). Tryptophan affects fish stress response differently depending on the circumstance. Tryptophan affects fish serotonergic activity and stress response. Tryptophan affects Adrenocorticotropic hormone (ACTH) and cortisol levels, and circulating cortisol is known as the most common indicator of stress in fish, which has been used to evaluate stress-mitigating effects of tryptophan in fish (Lepage *et al.*, 2002 and Hoseini *et al.*, 2012.). Effects of tryptophan on fish stress response are situation-dependent. Dietary tryptophan supplementation (0.68–2.72% of diet) has been found to inhibit chronic-stress effects on blood cortisol, glucose and immune response in *Labeorohita* and *Cirrhinus mrigala* (Ciji *et al.*, 2013). The effects of tryptophan on basal and post-stress cortisol show great variability in different fish species; as dietary tryptophan may increase (Lepage *et al.*, 2002; Hoseini and Hosseini, 2010) or decrease (Basic *et al.*, 2013a) basal cortisol levels or do not affect it (Herrero *et al.*, 2007). In addition, tryptophan may suppress (Lepage *et al.*, 2002; Hoseini *et al.*, 2012; Basic *et al.*, 2013) or not affect post-stress cortisol response (Basic *et al.*, 2013a).

The effects of dietary tryptophan on the physiological response of fish were found to be dependent on tryptophan levels, feeding duration and the nature of the stress (Lepage *et al.*, 2002; Hosseini and Hoseini, 2013). Exogenous tryptophan elevates serotonin levels in fish, and serotonin has been suggested to modulate the immune system (Duffy\_Whritenour and Zelikoff, 2008).

### **Hormone to Promote Growth in Fish**

Thyroid hormones have been well known to have an essential role in the early development of fish (Tagawa and Hirano, 1987) and have also been known to be present in fish eggs and newly hatched fish larvae (Tagawa and Hirano, 1990). These hormones change in the blood of female fish according to the reproductive cycle (Cyr *et al.*, 1988; Kang *et al.*, 1998). During ovarian maturation, hormones are transferred to the ovary and accumulate in oocytes. Injection of thyroid hormones increases the growth and survival rate of newly hatching larvae and has demonstrated beneficial effects in the development and survival of fish larvae (Ayson and Lam, 1993; Tachihara *et al.*, 1996). However, it should be noted that adverse effects of exogenous hormonal treatment also occurred in carp, *Cyprinus carpio* (Lam and Sharma, 1985), black seabream, *Acanthopagrus schlegeli* (Kang and Chang, 1997), and red drum, *Sciaenops ocellatus* (Moon *et al.*, 1994). The conflicting results may be associated with differences in the dose and mode of administration used with different species. Many studies report enhanced larval growth and survival by the transfer of exogenous thyroid hormone into offspring through the maternal circulation system: rabbit fish, striped bass, *Morone saxatilis* (Brown *et al.*, 1988), *Siganus guttatus* (Ayson and Lam, 1993), gold striped amberjack, *Seriola lalandi* (Tachihara *et al.*, 1996), and parrot fish, *Oplegnathus fasciatus* (El\_Zibdeh *et al.*, 1996).

The use of exogenous hormones in aquaculture aiming to induce spawning in different fish species in controlled condition and sex reversal in fishes where the mono sex culture in more profitable.



Additionally, many hormones used for promoting growth and reduce stress. These hormone administration helps to improve the production of the fisheries industry. However, it is advised that the use of hormones for different purpose should be regulated by the legal systems of the country that employ this technology, taking into account both human and environmental safety. The most common hormones to induce spawning includes ovaprim, ovatide, ovapel, GnRH and HCG. Generally, 17 $\alpha$ -methyl testosterone and 17 $\beta$ -estradiol used for sex reversal in many places. Tryptophan used to reduce stress and thyroid hormone to improve the growth rate of the fish. Therefore, while using hormones in fisheries sector utmost care must be taken to ensure food safety and human health.

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